

Volume 228, number 2, 351–355

FEB 05579

February 1988

## A new type of functional VIP receptor has an affinity for helodermin in human SUP-T1 lymphoblasts

Patrick Robberecht, Magali Waelbroeck, Philippe De Neef, Michèle Tastenoy, Philippe Gourlet, Jacqueline Cogniaux\* and Jean Christophe

*Department of Biochemistry and Nutrition, Medical School, Université Libre de Bruxelles, 115, Bd de Waterloo, B-1000 Brussels and \*Department of Virology, Institut Pasteur du Brabant, 608, rue Engeland, B-1180 Brussels, Belgium*

Received 26 December 1987

A new type of VIP receptor was characterized in human SUP-T1 lymphoblasts. The order of potency of unlabeled peptides, in the presence of [<sup>125</sup>I]helodermin, was: helodermin(1–35)-NH<sub>2</sub> = helodermin(1–27)-NH<sub>2</sub> > helospectin > VIP = PHI > [D-Ser<sup>2</sup>]VIP > [D-Asp<sup>3</sup>]VIP > [D-His<sup>1</sup>]VIP ≥ [D-Ala<sup>4</sup>]VIP > secretin = GRF. This specificity was distinct from that of all VIP receptors described so far in that: (i) the affinity for helodermin (K<sub>d</sub> = 3 nM) was higher than that of VIP (K<sub>d</sub> = 15 nM) and PHI (K<sub>d</sub> = 20 nM); and (ii) position 4 played an important role in ligand binding. The labeled sites were likely to be functional receptors as adenylate cyclase in crude lymphoblastic membranes (200–10 000 × g pellets) was stimulated by peptides, in the presence of GTP, with the following order of potency: helodermin(1–35)-NH<sub>2</sub> > helodermin(1–27)-NH<sub>2</sub> > helospectin = VIP = PHI.

Helodermin receptor; Vasoactive intestinal peptide; (T-cell-derived culture line, SUP-T1, Human)

### 1. INTRODUCTION

The neuropeptide VIP regulates T immune responses. Indeed, human blood T-lymphocytes possess high-affinity VIP receptors (K<sub>d</sub> 0.24–0.47 nM) and a highly sensitive VIP-responsive adenylate cyclase system [1–6]. In mice the density of VIP-binding sites on T-cells from secondary lymphoid organs is much higher than in thymus which suggests that the expression of VIP receptors depends on T-cell maturation. In mouse and sheep, VIP and dibutyl cyclic AMP modulate the migration of T-lymphocytes [7–10]. Besides, VIP inhibits, probably via cyclic AMP, the proliferative response of T-lymphocytes to the mitogens Con A and phytohemagglutinin [7].

The human immunodeficiency virus (HIV), the causative agent of AIDS (acquired immune deficiency syndrome), penetrates human T<sub>4</sub><sup>+</sup> (CD<sub>4</sub><sup>+</sup>)

helper/inducer T-lymphocytes after binding of the viral surface gp120 env glycoprotein with the cell surface 60 kDa antigenic marker T<sub>4</sub> (CD<sub>4</sub>). This initiation of the infectious cycle is followed by virus endocytosis, reduced T-cell proliferative response, virus proliferation, and virus exocytosis by a process involving again fusion of gp120 with T<sub>4</sub> at the cell surface [11]. In T<sub>4</sub><sup>+</sup> cell cultures, HIV infection induces syncytium formation by cell-to-cell fusion through further gp120-T<sub>4</sub> interactions. The resulting multinucleated giant cells are composed of both infected and uninfected T<sub>4</sub><sup>+</sup> cells recruited because the gp120 protein on the budding virion binds T<sub>4</sub> receptors on uninfected cells. The syncytia balloon and the death of both infected and uninfected cells then follows [12–16].

Pert and co-workers [17,18] claimed recently that the continuous epitope ASTTTNYT of gp120 is the major attachment sequence to T<sub>4</sub> receptors in human T-lymphocytes. This octapeptide (called peptide T because of its high threonine content) shows 5 sequence similarities (A-T-NYT) with the 4–11 sequence of VIP so that the question arises

*Correspondence address:* J. Christophe, Department of Biochemistry and Nutrition, Medical School, Université Libre de Bruxelles, Bd. de Waterloo 115, B-1000 Brussels, Belgium

whether VIP receptors and the adenylate cyclase system are related to  $T_4$ . If this were the case, the lymphotropic properties of HIV might result from the VIP-mimetic properties of gp120. Indeed, Pert et al. observed that peptide T blocks the adsorption on the virion to its  $T_4$  receptor in human peripheral blood  $T_4$  cells and concluded that peptide T competes with gp120 for  $T_4$  binding because of the region in gp120 specified by peptide T. Unfortunately, the T peptide sequence used by Pert et al. corresponds to a highly variable region of gp120 and, when tested by Sodroski et al. [19], exerts no effect on gp120 binding to SUP-T1 cells, a childhood T-cell lymphoma cell line (SUP-T1 = Stanford University Pediatric T-cell line 1) established from a pleural effusion of a lymphoblastic lymphoma showing a chromosome 14 inversion due to a break on the long (q) arm near the proximal portion [20].

At the Pasteur Institute of Brabant, the same SUP-T1 cell line is utilized for testing syncytium formation after AIDS invasion. We therefore decided to characterize the VIP receptors of this cell line and observed the existence of a new type of receptor with an affinity for helodermin 5–7-times higher than that for VIP and PHI, our binding data on intact cells being compatible with the ability of the same peptides to stimulate adenylate cyclase in crude membranes. The lizard peptides helodermin and helospectin belong to the VIP-PHI-secretin-GRF family [21–23]. Heloder-

min-like material is also present in normal mammalian (including human) tissues [24,25], human medullary thyroid carcinomas [26] and human pancreatic endocrine tumors [27]. The present data on human SUP-T1 lymphoblasts were unexpected as the potency of helodermin binding to VIP receptors [21,28] (and also secretin receptors in the rat pancreas [29]) had thus far been found to be weaker than that of VIP or secretin.

## 2. MATERIALS AND METHODS

Human lymphoblasts (SUP-T1 cell line) were cultured at 37°C in RPMI medium supplemented with 5% fetal calf serum. Stock cultures were transferred every 2 days in 2 vols of fresh medium. Cells were harvested by centrifugation at  $600 \times g$  for 10 min and resuspended in assay buffer. This buffer was made of 100 mM Tris, 120 mM NaCl, 5 mM KCl, 1.2 mM  $MgSO_4$ , 15 mM  $CH_3COONa$ , 10 mM glucose, 1 mM EDTA and 1% bovine serum albumin, and the pH was adjusted to 7.4.

Binding studies were conducted at 37°C in a final volume of 0.12 ml in the presence of [ $^{125}I$ ]helodermin [28] and increasing concentrations of unlabeled peptides. The reaction was stopped by filtration through glass-fiber filters GF/C (Whatman, Maidstone, England) presoaked for 24 h in 0.05% polyethyleneimine in order to reduce nonspecific adsorption of tracer to the filter. Nonspecific binding was defined as binding in the presence of 1  $\mu$ M unlabeled helodermin.

To prepare crude membranes, lymphoblasts were pelleted at 20°C, resuspended in 1 mM  $NaHCO_3$  (pH 7.0) then dispersed in liquid nitrogen. After thawing and centrifugation at  $200 \times g$  for 10 min, the supernatant was centrifuged at  $10000 \times g$  for 15 min at 4°C. The final pellet was resuspended in the same volume of homogenization medium and either tested immediately or stored at -80°C until use.

Adenylate cyclase activity was determined as previously

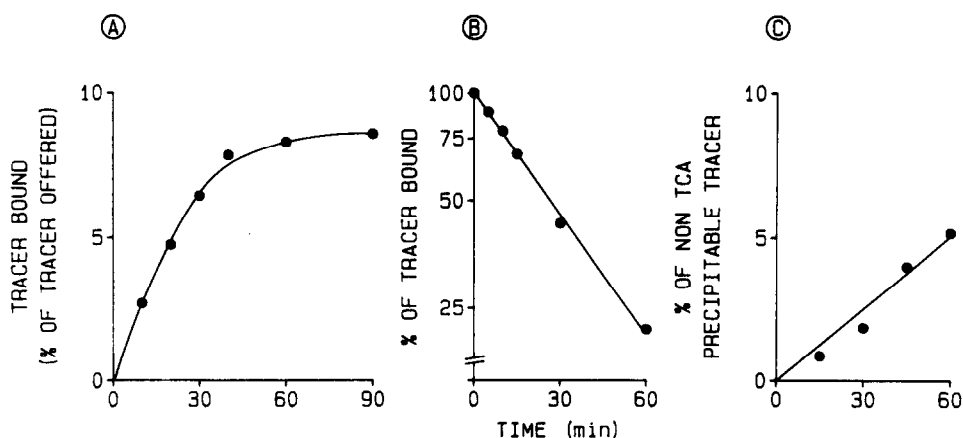


Fig.1. Kinetics of association (A), dissociation (B) and degradation (C) of [ $^{125}I$ ]helodermin in the presence of SUP-T1 lymphoblasts. Experiments were conducted at 37°C as described in section 2. All results were the means of experiments performed in duplicate on three different preparations.

described [30]. Under all conditions tested cyclic AMP production was linear during the incubation period.

Natural helodermin and natural helospectin were prepared as previously reported [31] from lizard venoms (Sigma, St Louis, MO), synthetic helodermin was provided by Dr J.P. Durieux (Novabiochem, Läufelfingen, Switzerland), synthetic helodermin analogues and fragments were gifts from Dr N. Yanaihara (Shizuoka College of Pharmacy, Shizuoka, Japan) and VIP, PHI, GRF and VIP analogues were prepared by Dr D.H. Coy (Tulane University School of Medicine, New Orleans, LA). Secretin was prepared by Dr W. König (Hoechst, Frankfurt am Main, FRG). All other reagents were of the highest grade available.

### 3. RESULTS

The specific binding of [ $^{125}$ I]helodermin to SUP-T1 cells reached pseudoequilibrium after 60 to 90 min (fig.1A). Nonspecific binding represented 20% of total binding. The dissociation rate of bound tracer was examined by chemical dilution after washing the labeled cells and their resuspension in 10 vols of fresh buffer. The monophasic dissociation of the radioligand ( $t_{1/2} = 22 \pm 5$  min, mean  $\pm$  SE of 3 determinations) suggested that the tracer labeled a single receptor population (fig.1B). Tracer degradation was extremely low as only 5% of the radioactivity offered was no longer precipitable by 10% trichloroacetic, after a 60 min incubation at 37°C in the presence of cells (fig.1C). Further experiments were therefore conducted over a 60 min incubation period.

[ $^{125}$ I]Helodermin binding was completely inhibited by unlabeled helodermin ( $K_d = 3$  nM), VIP ( $K_d = 15$  nM) and PHI ( $K_d = 20$  nM). Secretin and GRF were virtually ineffective (fig.2). The fragment helodermin(1-27)-NH<sub>2</sub> and synthetic helodermin(1-35)-NH<sub>2</sub> were equipotent to natural helodermin (fig.3, and not shown). Natural helospectin was slightly less potent than helodermin ( $K_d = 10$  nM) but more potent than VIP. The fragment helodermin(7-35)-NH<sub>2</sub> recognized poorly the receptor and all other helodermin fragments tested were ineffective (fig.3). Four VIP analogues inhibited [ $^{125}$ I]helodermin binding (fig.4) with the following order of decreasing potency: VIP > [D-Ser<sup>2</sup>]VIP > [D-Asp<sup>3</sup>]VIP > [D-His<sup>1</sup>]VIP  $\geq$  [D-Ala<sup>4</sup>]VIP. This order differed clearly from that found for VIP receptors in rat pancreatic, hepatic and pituitary membranes [30]: VIP > [D-Ala<sup>4</sup>]VIP > [D-Asp<sup>3</sup>]VIP > [D-Ser<sup>2</sup>]VIP > [D-His<sup>1</sup>]VIP.

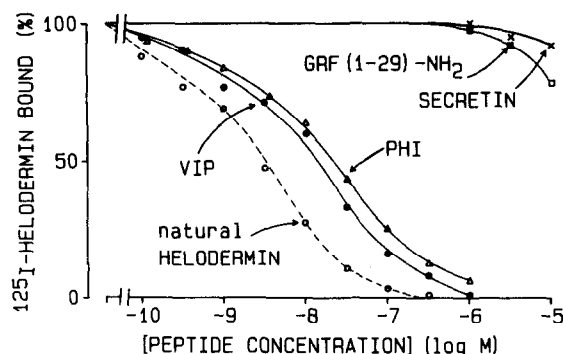


Fig.2. Inhibition of [ $^{125}$ I]helodermin binding to SUP-T1 lymphoblasts by natural helodermin, VIP, PHI, secretin and human GRF(1-29)-NH<sub>2</sub>. Incubation was conducted at 37°C during 60 min. Results are expressed in % of tracer bound and were the means of experiments performed in duplicate on three different preparations.

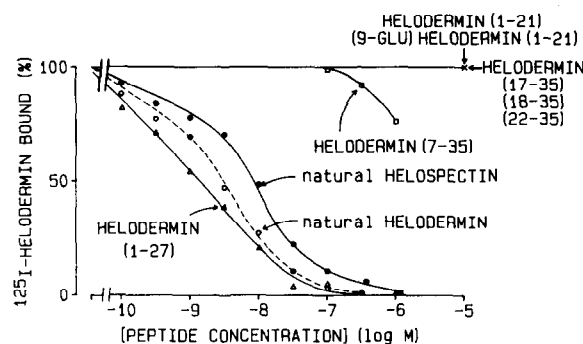


Fig.3. Inhibition of [ $^{125}$ I]helodermin binding to SUP-T1 lymphoblasts by helodermin, helospectin and helodermin fragments (all peptides were amidated). Same methodology and representation as in fig.2.

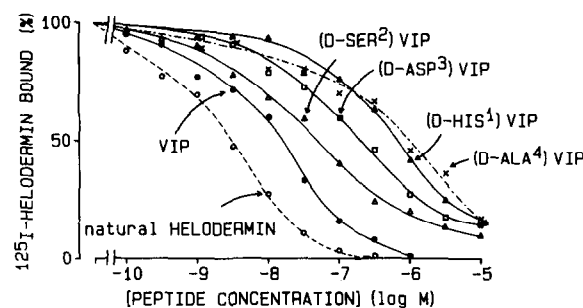


Fig.4. Inhibition of [ $^{125}$ I]helodermin binding to SUP-T1 lymphoblasts by helodermin, VIP and the VIP analogues [D-His<sup>1</sup>]VIP, [D-Ser<sup>2</sup>]VIP, [D-Asp<sup>3</sup>]VIP, and [D-Ala<sup>4</sup>]VIP. Same methodology and representation as in figs 2 and 3.

Helodermin(1-35)-NH<sub>2</sub> and (1-27)-NH<sub>2</sub>, helospectin, VIP and PHI stimulated the adenylate cyclase activity of crude membranes from SUP-T1 lymphoblasts in the presence of 10  $\mu$ M GTP (fig.5). VIP and PHI were slightly less effective than the other peptides.

#### 4. DISCUSSION

Our results demonstrated the presence, in human T-lymphoblasts (SUP-T1), of receptors capable of recognizing peptides of the VIP family with a selectivity that had not previously been observed. Indeed, the decreasing order of potency of these peptides (figs 2-4) was: heloder-

min(1-35)-NH<sub>2</sub> = helodermin(1-27)-NH<sub>2</sub> > helospectin > VIP = PHI > [D-Ser<sup>2</sup>]VIP > [D-Asp<sup>3</sup>]VIP > [D-His<sup>1</sup>]VIP  $\geq$  [D-Ala<sup>4</sup>]VIP  $\gg$  secretin = GRF, implying a unique selectivity pattern when compared to that found in all other VIP receptors previously characterized [21,28-30]. The term 'helodermin-affinity receptor' can thus be proposed in this case. It is of interest to note that the amidated fragment helodermin(1-27)-NH<sub>2</sub> was as active as helodermin(1-35)-NH<sub>2</sub> but that the shorter amidated fragment helodermin(1-21)-NH<sub>2</sub> had completely lost the ability to recognize the receptors. However, the C-terminal sequence of helodermin(1-27) was not the only portion of the molecule involved in receptor recognition as helodermin(7-35)-NH<sub>2</sub> showed very weak affinity only. As for the other peptides of the family, the whole molecule was probably required for proper receptor occupancy. Position 4 of the peptide was of particular interest in distinguishing between helodermin-affinity receptors and VIP receptors: [D-Ala<sup>4</sup>]VIP was 100-fold less potent than VIP on the present helodermin-affinity receptors (fig.4) while being only 2-fold less potent than VIP on several VIP receptors [30].

[<sup>125</sup>I]Helodermin binding sites in intact cells were likely to be functional receptors as the apparent  $K_d$  of helodermin(1-35)-NH<sub>2</sub>, helodermin(1-27)-NH<sub>2</sub>, helospectin(1-37), VIP and PHI, required for half-maximal occupancy of these sites, correlated reasonably well with the concentrations needed for half-maximal adenylate cyclase activation in crude membranes, in the presence of GTP.

The presence of receptors for the VIP family of peptides on SUP-T1 cells derived from a T-cell lymphoma is not surprising as VIP receptors are present in human peripheral T-cell lymphocytes [1-6]. VIP also binds to another human lymphoblastoid cell line (Molt 4b T, suppressor). The affinity of normal lymphocytic receptors for VIP ranges between 0.24 and 0.47 nM whereas that of Molt 4b T-lymphoblasts is 7.0-15 nM [32,33], a value comparable to that observed by us in SUP-T1 cells. The helodermin-affinity receptors of SUP-T1 cells differ, however, from Molt 4b T-lymphoblast VIP receptors based on the relative potency of PHI and GRF. It is tempting to suggest that the new type of receptor in human SUP-T1 lymphoblasts is either a transformed VIP receptor

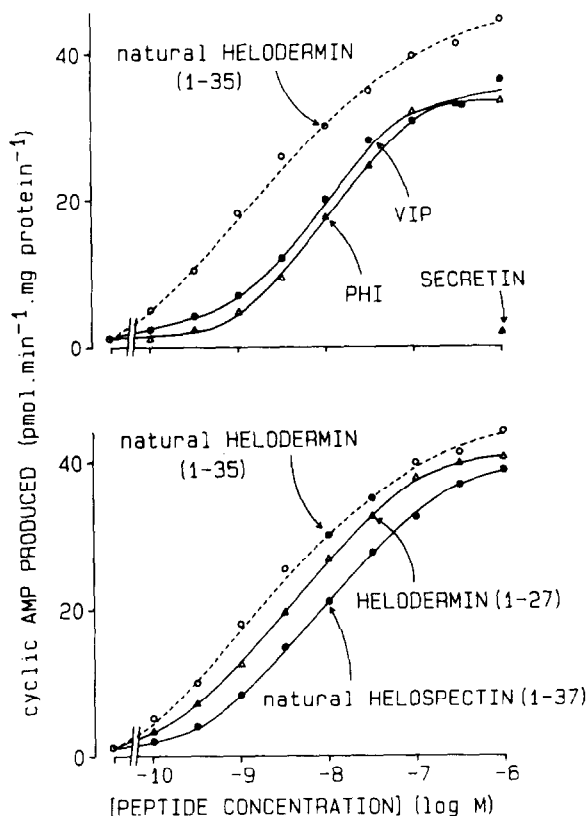


Fig.5. Effect of increasing concentrations of helodermin(1-35)-NH<sub>2</sub>, helodermin(1-27)-NH<sub>2</sub>, helospectin(1-37), VIP and PHI on adenylate cyclase activity in crude membranes from SUP-T1 lymphoblasts. The results were expressed in pmol cyclic AMP produced  $\cdot$  min<sup>-1</sup>  $\cdot$  mg protein<sup>-1</sup>. The data were the means of experiments performed in duplicate on three different preparations.

or a helodermin receptor revealed by malignancy that has not (yet) been identified in healthy tissues.

*Acknowledgements:* Aided by grant 3.4571.85 from the Fonds de la Recherche Scientifique Médicale (Belgium) and grant STJ-0001-1-B from the Commission des Communautés Européennes (EEC).

## REFERENCES

- [1] Guerrero, I.M., Prieto, J.C., Elorza, F.L., Ramirez, R. and Goberna, R. (1981) *Mol. Cell. Endocrinol.* 21, 151–160.
- [2] Danek, A., O'Dorisio, M.S., O'Dorisio, T.M. and George, J.M. (1983) *J. Immunol.* 131, 1173–1177.
- [3] O'Dorisio, M.S., Hermina, N.S., O'Dorisio, T.M. and Balcerzak, S.P. (1981) *J. Immunol.* 127, 2551–2554.
- [4] Ottaway, C.A., Bernaerts, C., Chan, B. and Greenberg, G.R. (1983) *Can. J. Physiol. Pharmacol.* 61, 664–671.
- [5] O'Dorisio, M.S. (1987) *Fed. Proc.* 46, 192–195.
- [6] O'Dorisio, M.S., Wood, C.L. and O'Dorisio, T.M. (1985) *J. Immunol.* 135, 792s–796s.
- [7] Ottaway, C.A. (1984) *J. Exp. Med.* 160, 1054–1059.
- [8] Ottaway, C.A. and Greenberg, G.R. (1984) *J. Immunol.* 132, 417–423.
- [9] Moore, T.C. and Lachmann, P.J. (1982) *Immunology* 47, 423–428.
- [10] Moore, T.C. (1984) *Immunology* 52, 511–518.
- [11] Dagleish, A.G., Beverley, P.C.L., Clapham, P.R., Crawford, D.H., Greaves, M.F. and Weiss, R.A. (1984) *Nature* 312, 763–767.
- [12] Ho, D.D., Pomerantz, R.J. and Kaplan, J.C. (1987) *N. Engl. J. Med.* 317, 278–286.
- [13] Lifson, J.D., Reyes, G.R., McGrath, M.S., Stein, B.S. and Engleman, E.G. (1986) *Science* 232, 1123–1127.
- [14] Lifson, J.D., Feinberg, M.B., Reyes, G.R., Rabin, L., Banapour, B., Chakrabarti, S., Moss, B., Wong-Staal, F., Steimer, K.S. and Engleman, E.G. (1986) *Nature* 323, 725–728.
- [15] Sodroski, J., Goh, W.C., Rosen, C., Campbell, K. and Haseltine, W.A. (1986) *Nature* 322, 470–474.
- [16] Yoffe, B., Lewis, D.E., Petrie, B.L., Noonan, C.A., Melnick, J.L. and Hollinger, F.B. (1987) *Proc. Natl. Acad. Sci. USA* 84, 1429–1433.
- [17] Pert, C.B., Hill, J.M., Ruff, M.R., Berman, R.M., Robey, W.G., Arthur, L.O., Ruscetti, F.W. and Farrar, W.L. (1986) *Proc. Natl. Acad. Sci. USA* 83, 9254–9258.
- [18] Ruff, M.R., Martin, B.M., Ginns, E.I., Farrar, W.L. and Pert, C.B. (1987) *FEBS Lett.* 211, 17–22.
- [19] Sodroski, J., Kowalski, M., Dorfman, T., Basiripour, L., Rosen, C. and Haseltine, W. (1987) *Lancet*, 1428–1429.
- [20] Hecht, F., Morgan, R., Kaiser-McCaw Hecht, B. and Smith, S.D. (1984) *Science* 226, 1445–1447.
- [21] Robberecht, P., Waelbroeck, M., Dehay, J.P., Winand, J., Vandermeers, A., Vandermeers-Piret, M.-C. and Christophe, J. (1984) *FEBS Lett.* 166, 277–282.
- [22] Hoshino, M., Yanaihara, C., Hong, Y.A., Kishida, S., Katsumaru, Y., Vandermeers, A., Vandermeers-Piret, M.-C., Robberecht, P., Christophe, J. and Yanaihara, N. (1984) *FEBS Lett.* 178, 233–239.
- [23] Parker, D.S., Raufman, J.-P., O'Donohue, T.L., Bledsoe, M., Yoshida, H. and Pisano, J.J. (1984) *J. Biol. Chem.* 259, 11751–11755.
- [24] Robberecht, P., De Graef, J., Woussen-Colle, M.-C., Vandermeers-Piret, M.-C., Vandermeers, A., De Neef, P., Cauvin, A., Yanaihara, C., Yanaihara, N. and Christophe, J. (1985) *Biochem. Biophys. Res. Commun.* 130, 333–342.
- [25] Robberecht, P., De Neef, P., Vandermeers, A., Vandermeers-Piret, M.-C., Svoboda, M., Meuris, S., De Graef, J., Woussen-Colle, M.-C., Yanaihara, C., Yanaihara, N. and Christophe, J. (1985) *FEBS Lett.* 190, 142–146.
- [26] Sundler, F., Christophe, J., Robberecht, P., Yanaihara, N., Yanaihara, C., Grunditz, T. and Håkanson, R. (1988) *Regul. Pept.*, in press.
- [27] Robberecht, P., Vandermeers, A., Vandermeers-Piret, M.-C., Gourlet, P., Cauvin, A., De Neef, P. and Christophe, J. (1988) *Ann. NY Acad. Sci.*, in press.
- [28] Robberecht, P., Waelbroeck, M., De Neef, P., Camus, J.C., Vandermeers, A., Vandermeers-Piret, M.-C. and Christophe, J. (1984) *FEBS Lett.* 172, 55–58.
- [29] Dehay, J.P., Winand, J., Damien, C., Gomez, F., Poloczek, P., Robberecht, P., Vandermeers, A., Vandermeers-Piret, M.-C., Stiévenart, M. and Christophe, J. (1986) *Am. J. Physiol.* 251, G602–G610.
- [30] Robberecht, P., Coy, D.H., De Neef, P., Camus, J.C., Cauvin, A., Waelbroeck, M. and Christophe, J. (1986) *Eur. J. Biochem.* 159, 45–49.
- [31] Vandermeers, A., Gourlet, P., Vandermeers-Piret, M.-C., Cauvin, A., De Neef, P., Rathé, J., Svoboda, M., Robberecht, P. and Christophe, J. (1987) *Eur. J. Biochem.* 164, 321–327.
- [32] Beed, E.A., O'Dorisio, M.S., O'Dorisio, T.M. and Gaginella, T.S. (1983) *Regul. Pept.* 6, 1–12.
- [33] Wood, C.L. and O'Dorisio, M.S. (1985) *J. Biol. Chem.* 260, 1243–1247.